Remarks

Applicants have canceled Claims 1-4 and 28-40. Claim 5, which depended on Claim 1, has been amended to become an independent claim. The amendment does not introduce new matter, but incorporates the compound claimed in canceled Claim 1 into the text of Claim 5. Entry of this amendment is respectfully requested.

The Examiner rejected Claims 5-27 under 35 USC 112, second paragraph. Specifically, the Examiner stated, with regard to Claim 10, that the terms "known concentration of the membrane/radioligand mixture", "the final concentration of the membrane containing I_{Kr} channel is predetermined" are unclear, that the term IC_{50} is undefined, that the moieties which are "isolated" and "measured" in Steps 4) and 5) are unclear, and that the claim does not correlate calculation of the IC_{50} values with assessment of the binding of the test compound. The Examiner also mentioned that Claim 5 does not correlate the step of monitoring whether the test compound influences the binding of the radioligand compound to the membrane containing the I_{Kr} channel, with assessment of the I_{Kr} channel blocker activity of the test compound.

Applicants have amended Claim 10 by deleting the phrases "known concentration" and "final concentration.... is predetermined" from step 3. Applicants maintain that step 3 of amended Claim 10 particularly points out the step of incubating a quantity of the membrane/radioligand mixture with a solution of test compound, control vehicle or compound of Formula II.

"the IC_{50}" means the concentration of test compound that provides 50% inhibition of binding of the radioligand compound to the membrane. As mentioned on page 4, lines 20-21, determination of this value allows one to quantify the potency of compound interaction with the I_{Kr} channel. IC_{50} corresponds to the inflection point of a non-linear 4 parameter regression plot based on inhibition as a function of concentration. The value is a measure of a compound's affinity of interaction with the ERG channel (see page 4, lines 29-32).

Applicants have amended Claim 10, steps 4 and 5, so that it correlates with step 7. These isolation and measurement steps allow for a comparison of the [^{35}S]-radioligand counts bound to the membranes in the presence of a solvent vehicle (100% control), in the presence of 1 μM Formula II (final non-specific binding) and at each of the different concentrations of the test compound (see page 4, lines 21-24).

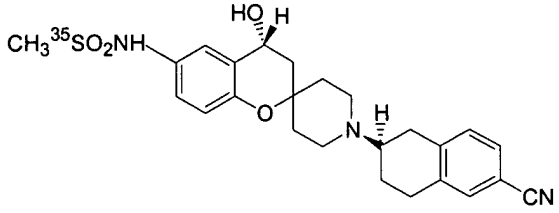
Claim 10 has been amended to correlate calculation of the IC_{50} with assessment of the binding of the test compound.

Claim 5 has been amended to correlate the step of monitoring whether the test compound influences the binding of the radioligand compound to the membrane containing the I_{Kr} channel, with assessment of the I_{Kr} channel blocker activity of the test compound.

In view of these comments and the amendments of Claims 5 and 10, applicants respectfully request reconsideration and withdrawal of the rejection of Claims 5-27.

The Examiner rejected Claims 5-27 under 35 USC 103(a) as unpatentable over Baldwin et al, in combination with each of Chadwick et al., Fiset et al., Geonzon et al., or Duff et al., and with Dean et al. The Examiner indicated that Baldwin et al. describes the non-radiolabeled, sulfonamide compound of formula I of Claim 1, that each of Chadwick et al., Fiset et al., Geonzon et al., and Duff et al. describe assays that assess the membrane K+ channel blocking activity of radiolabeled [3H] dofetilide, and that Dean et al. describe use of 35 S-containing sulfonamide groups for sulfonamide-containing ligands used in receptor binding radioassays.

Applicant maintains that the method claimed in Claims 5-27, which employs the sulfur-35 radiolabeled compound.



to assess binding of a test compound to a membrane containing the I_{Kr} channel, is not obvious.

Determination of the appropriate structure of a radiolabeled compound for assessing the binding of a test compound to a membrane containing the I_{Kr} channel requires consideration of compound stability and preparation convenience.

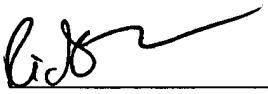
Sulfur-35, tritium and iodine-125 are all used to radiolabel compounds. Iodine-125, for example, is commonly used for methods in which specific activity >100 Ci/mmol are required. However, in the I_{Kr} channel assay, introduction of the iodine atom severely compromises binding affinity at the targeted protein, and adversely affects physiochemical properties. Introduction of tritium does not provide the high specific radioactivity that is obtained from sulfur-35 (~90 Ci/mmol for tritium vs >1000 Ci/mmol for

sulfur-35). We have found that sulfur-35 provides the high specific activity (>100 Ci/mmol) that is essential for an assay with adequate sensitivity to support the high throughput required for screening activities against the I_{Kr} channel. This radiolabeled compound is not obvious in view of what is known in the art. Reconsideration and withdrawal of the rejection of Claims 5-27 under 35 USC 103(a) is respectfully requested.

In view of the cancellation of Claims 1-4 and 28-40, amendments of Claims 5 and 10, and above remarks, applicants believe the application is in condition for allowance.

Respectfully submitted,

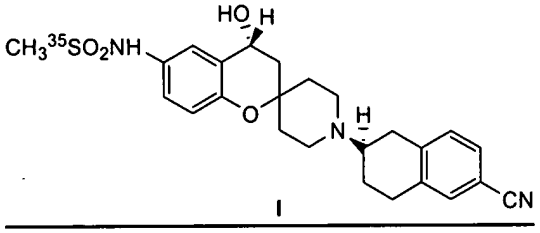
Date: July 18, 2003



Richard S. Parr
Attorney for Applicants
MERCK & CO., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907
Tel.: (732) 594-4958

AMENDMENTS WITH MARKINGS TO SHOW CHANGES MADE

5. (amended) A method for characterizing the activity of a compound as an I_{Kr} channel blocker comprising contacting the test compound with a membrane containing the I_{Kr} channel in the presence of the radioligand compound ~~of Claim 1~~



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or a pharmaceutically acceptable salt thereof,

~~and~~ monitoring whether the test compound influences the binding of the radioligand compound to the membrane containing the I_{Kr} channel, and determining the I_{Kr} channel blocker activity of the test compound.

10. (amended) A method for assessing the binding of a test compound to a membrane containing the I_{Kr} channel using a radioligand compound of Formula I, [^{35}S]-radiolabeled (+)-N-[1'-(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydoxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-methanesulfonamide, comprising the steps of:

1) preparing solutions of the test compound at 5 or more different concentrations, a solution of control vehicle and a solution of (+)-N-[1'-(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydoxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-methanesulfonamide (compound of Formula II) in a solvent;
2) mixing the radioligand compound of Formula I with the membrane containing the I_{Kr} channel diluted with an assay buffer to form a membrane/radioligand mixture of known concentration;
3) incubating a quantity of ~~known concentration of~~ the membrane/radioligand mixture with the solution of test compound, control vehicle or compound of Formula II, as recited in Step 1, for a set time period at a temperature range of between about 4°C and about 37°C to give a mixture of membrane bound with the radioligand and the test compound, the control vehicle or the compound of Formula II~~, where the final concentration of the membrane containing the I_{Kr} channel is predetermined~~;

4) isolating from the incubated mixture the membrane bound with the radioligand and the test compound, the membrane bound with the radioligand and with the control vehicle or the membrane bound with the radioligand and the compound of Formula II;

5) measuring the radioactivity of the isolated membrane bound with the radioligand and the test compound, the membrane bound with the radioligand and with the control vehicle or the membrane bound with the radioligand and the compound of Formula II;

6) repeating steps 3 through 5 with the test compound at each concentration, the solution of control vehicle and the solution of the compound of Formula II, as recited in Step 1; and

7) calculating the IC_{50} corresponding to the measured radioactivity of: 1) the membrane bound with the radioligand and each concentration of the test compound, 2) the membrane bound with the radioligand and with the control vehicle, and 3) the membrane bound with the radioligand and the compound of Formula II, to assess the binding of the test compound to the membrane.